

AD _____

Award Number: W81XWH-05-1-0181

TITLE: Activation and Protection of Dendritic Cells in the Prostate Cancer Environment

PRINCIPAL INVESTIGATOR: Georgi Guruli, M.D., Ph.D.
Mark L. Jordan, M.D.

CONTRACTING ORGANIZATION: University of Medicine and Dentistry of
New Jersey
Newark, NJ 07107-3001

REPORT DATE: February 2007

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.					
1. REPORT DATE 01-02-2007		2. REPORT TYPE Annual		3. DATES COVERED 15 Jan 2006 – 14 Jan 2007	
4. TITLE AND SUBTITLE Activation and Protection of Dendritic Cells in the Prostate Cancer Environment				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER W81XWH-05-1-0181	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Georgi Guruli, M.D., Ph.D. Mark L. Jordan, M.D.				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) University of Medicine and Dentistry of New Jersey Newark, NJ 07107-3001				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT First annual report for this award. Experiments were conducted as was scheduled in the Statement of Work. So far studies have demonstrated for the first time the presence of endothelin receptors on murine DC, and the fact of endothelin-1 production by murine DC upon stimulation with TNF•. Phenotyping of dendritic cells stimulated with TNF• and treated with endothelin receptor inhibitors demonstrated decreased expression of pro-inflammatory co-stimulatory molecules (CD40, VD80, CD86, CD205, MHC class II) with the blockade of ETA receptors, and no change or mild increase in the expression of co-stimulatory molecules with the blockade of ETB receptors. In vivo administration of endothelin A receptor inhibitor abolished the effect of infectious stimulus to mobilize dendritic cells to draining lymph nodes. Functional studies are under way to further characterize the role of endothelin receptors in the biology of dendritic cells, as well as to study the interaction of dendritic cells and prostate cancer cells, and develop the means of active cell therapy for murine prostate cancer model.					
15. SUBJECT TERMS Prostate cancer, Dendritic cells, Endothelin receptors, Immunotherapy					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	26	19b. TELEPHONE NUMBER (include area code)

Table of Contents

Introduction.....	4
Body.....	4
Key Research Accomplishments.....	6
Reportable Outcomes.....	7
Conclusions.....	7
References.....	7
Appendices.....	8

Introduction:

This study is being conducted for the (i) characterization of the prostate cancer and dendritic cells (DC) interaction; (ii) defining the role of endothelin axis in the maturation of DC, (iii) elucidating the role of endothelin axis in the prostate cancer-DC interaction, and (iv) modification of dendritic cells to be used in the treatment of prostate cancer. Mouse model will be used. This is the report for the first 2 years of the award. Experiments are progressing according to the plan so far. Experiments reported during the first annual report will be discussed briefly during this submission.

Report:

First task was to proceed with the characterization of role of endothelin axis in DC. For this purpose, DC were grown from C57BL/6 mice bone marrow, as was described earlier¹. Briefly, bone marrow cells were first depleted of RBC with lysing buffer for 2–3 min. The single-cell suspensions then was incubated with a cocktail of Abs (α CD4, α CD8a, and B220) for 1 h at 4°C, followed by incubation with rabbit complement for 30 min at 37°C to deplete cells expressing lymphocyte Ags B220, CD4, and CD8. Cells were then incubated overnight (37°C, 5% CO₂) in six-well plates (Falcon, Franklin Lakes, NJ) at a concentration of 10⁶ cells/ml in complete medium, consisting of RPMI 1640, 2 mM L-glutamine, 50 µg/ml gentamicin sulfate, 10 mM HEPES, 10% FBS, 0.1 mM nonessential amino acids, and 1 mM sodium pyruvate (Life Technologies). The nonadherent cells were collected by gentle pipetting and resuspended at a concentration of 2.5 x 10⁵ cells/ml in complete medium supplemented with 1000 U/ml recombinant murine GM-CSF and recombinant murine IL-4 (R&D system). Cells were cultured in six-well plates (4 ml/well) for 7 days at 37°C in 5% CO₂. Nonadherent DC are collected by gentle pipetting, counted, characterized as described previously², and used for further studies.

For the characterization of the general impact of endothelin receptors, dendritic cells were stimulated with TNF α for the endothelin production and the expression of endothelin receptors, since our preliminary data indicated increased expression of endothelin receptors upon stimulation in mice (unpublished data). We have previously demonstrated as well increased production of endothelin-1 (ET-1) by human DC, and increased expression of endothelin receptors³.

For the characterization of ET-1 production, DC were cultured as described above, and stimulated with TNF α (10ng/ml, added on Day 5) for 48 hours. After that, supernatant was collected, and ET-1 was measured using ET-1 ELISA kit as described³. Stimulated cells produced 1044.2 \pm 118.6 pg/ml/mln cells, while nonstimulated cells produced 351.9 \pm 131.9 pg/ml/mln cells. The difference was statistically significant (P=0.008).

Next, changes in phenotype has been evaluated. Murine DC has been stimulated with TNF α on day 5 for 48 hours. Stimulated DC were treated with endothelin receptor inhibitors BQ-123 (Selective ET_A receptor inhibitor, American Peptide Company), at a final concentration of 10⁻⁶ M, for the last 48 hours, and with BQ-788 (Selective ET_B receptor inhibitor, American Peptide Company), at a final concentration of 10⁻⁶ M, for the last 48 hours as well. After that, cells were collected, washed, counted and stained for flow cytometry. We have evaluated cells for the expression of CD40, CD80, CD86, MHC class II antigen, and CD205. Briefly, stimulation with TNF α resulted in the increased expression

of these costimulatory molecules (as expected). The blockade of ET_A receptor with BQ-123 induced in general decreased expression of the costimulatory molecules, which was especially significant for CD40 and CD205 (difference was statistically significant by chi-square test, $P < 0.001$). On the other hand, the blockade of ET_B receptor with BQ-788 resulted in no change or increased expression of costimulatory molecules.

For the further characterization of the endothelin axis on dendritic cells, we proceeded with the mixed leukocyte reaction (MLR). Briefly, allogeneic T cells were generated from balb mice spleens using murine T cell enrichment columns (R&D Systems, Minneapolis, MN). Isolated T cells were placed in the round-bottom 96-well plates, 3×10^5 per well, and DC were added with decreasing concentrations. After 72 hours of incubation at 37°C, ³H-TdR (New England Nuclear Co., Boston, MA) was added to the DC/T cell mixture, 1 μCi per well. T cell proliferation was measured by the ³H-TdR uptake in 16 hours. Cells were harvested onto glass fiber filter paper with a semi-automated microharvester, and ³H-TdR incorporation was determined by liquid scintillation spectroscopy. There were four experimental groups: 1) DC prepared as usual; 2) DC treated with TNFα during the last 48 hours; 3) DC treated with TNFα and BQ-123 for the last 48 hours, and 4) DC treated with TNFα and BQ-788 for the last 48 hours. Preliminary results of the experiment are presented in the Figure 2. As it can be seen, addition of TNFα resulted in increased ability of the DC to stimulate T cells. While the addition of the BQ-788 didn't produce significant changes in these preliminary experiments, addition of BQ-123 resulted in decreased ability of DC to stimulate T cells, in comparison to DC treated with TNFα alone. These results lead us to speculate that the stimulation of ET_A receptors may lead to the activation of DC, and that their blockade might abolish or lessen immune response.

During the next set of experiments, the influence of prostate cancer cells on DC was evaluated. DC were grown as described above. On days 5 and 6, 1 ml of RM-1 (murine prostate cancer cells) cells supernatant was added to each well (in the 6-well plates). Control DC received media only (1 ml per well on days 5 and 6). On day 7, cells were collected, and flow cytometry was performed for the expression of costimulatory molecules. Results are presented on Figure 3. Addition of tumor cells supernatant resulted in the 10% drop in the expression on CD80 marker, the expression of CD205 was reduced significantly as well. More experiments are under way, including the functional evaluation of DC after their co-incubation with prostate cancer cells.

In vivo experiments were performed as well. Tumors were induced by subcutaneous injection of 25,000 RM-1 murine prostate cancer cells into groups (n=5) of the C57BL/6 mice. When tumors became palpable (day 6), treatment was initiated with injection of 1.5×10^6 bone marrow derived DC into the tumor. Group 1 received Hank's solution (control); Group 2 – unmodified DC; Group 3 – DC treated with TNF-α during the last 48 hours; Group 4 - DC treated with TNF-α and ET_B receptor antagonist BQ-788 during the last 48 hours (our previous studies have shown the increased expression of endothelin receptors after the stimulation of DCs with TNF-α, and improved DC survival with the blockade of ET_B receptors). Two further injections were performed on days 9 and 12. Tumor size was assessed starting from day 14 until animal sacrifice. By day 24, mean tumor size reached $1796 \pm 166 \text{ mm}^3$ in the Group 1 (control), $1556 \pm 186 \text{ mm}^3$ in the Group 2, $1508 \pm 166 \text{ mm}^3$ in the group 3, and $397 \pm 186 \text{ mm}^3$ in Group 4 ($P < 0.001$ versus

control). Difference in mean tumor size became significant starting from day 20 (figures 4 and 5).

One gene array experiment was performed, to assess the influence of prostate cancer cells on DC. Briefly, 7-day-old cultured DC were harvested and co-incubated with the murine prostate cancer cell line RM-1 in six-well plates. DC and tumor cells were separated using membrane inserts with 0.4- μ m pore size, which exclude direct cell-to-cell contact, but allow free exchange of soluble factors. Specifically, 5×10^5 DC will be placed in six-well plates in 3 ml of medium. One million prostate cancer cells resuspended in 2 ml of medium were placed into the inserts on the top of each well. As controls, DC were co-incubated with murine splenocytes. DC were harvested 48 h later, washed, RNA was extracted using RNA extraction minikit, and used for gene arrays. We used mouse 22K Oligo Arrays (Center for Applied Genomics) which is composed of fifteen-thousand 70 mer oligonucleotides corresponding to specific mouse transcripts. The oligonucleotides were spotted onto poly-lysine-coated glass microscope slides by using a Gene Machines Omnigrid 100 arrayer (Genomic Solutions, Ann Arbor, Mich.) and SMP3 pins (Telechem, Sunnyvale, Calif.). RNA labeling and hybridization was performed using the 3DNA Array detection Array 350 Kit (Genisphere Inc.) according to the manufacturer's instructions. We used "comparison design" for this experiment, where RNA's were compared to each other directly, without standard. Preliminary analyze of data demonstrated so far decreased expression of receptors for IL-12 and interferon gamma in DC incubated with RM-1 cells. More experiments with "reference settings" are scheduled.

Key research Accomplishments:

- Production of ET-1 by murine DC has been documented first time, as well as the presence of endothelin receptors on murine DC.
- The influence of endothelin receptor inhibitors on DC phenotype was demonstrated. Functional experiments (MLR) demonstrated the possible involvement of the ET_A receptors in the activation of DC, driving them towards TH1 response. It seems that ET_B receptor stimulation might drive DC toward tolerance, with decreased expression of co-stimulatory molecules. Further studies are needed to clarify the exact role these receptors in DC biology.
- Treatment of DC with prostate cancer cells supernatants induced decreased expression of some co-stimulatory molecules.
- Treatment of murine prostate cancer by intratumoral injection of the modified dendritic cells resulted in the reduction of the tumor growth. These data may provide basis for the development of clinical trials protocol.

Reportable outcome:

Research data have been submitted and accepted for the presentation at the AUA 2007 and AACR 2007 annual Meetings. Final copy of the abstract is attached.

Conclusion:

So far experiments have demonstrated the possible role of endothelin receptor inhibitors in the function of DC, which can be useful in the treatment of different diseases, ranging from cancer to transplantation. Our in vivo experiments showed the possible role of endothelin receptors modification on DC in the treatment of prostate cancer in mice. More experiments are underway, and clinical trials protocol is being planned for patients with advanced prostate cancer, using modified autologous DC.

References:

1. Pirtskhalaishvili G, Shurin GV, Gambotto A, Esche C, Wahl M, Yurkovetsky ZR, Robbins PD, Shurin MR. Transduction of dendritic cells with Bcl-xL increases their resistance to prostate cancer-induced apoptosis and antitumor effect in mice. *Journal of Immunology*. 2000;165:1956-1964
2. Shurin MR, Pandharipande PP, Zorina TD, Haluszczak C, Subbotin VM, Hunter O, Brumfield A, Storkus WJ, Maraskovsky E, Lotze MT. FLT3 ligand induces the generation of functionally active dendritic cells in mice. *Cell Immunol*. 1997;179:174-184
3. Guruli G, Pflug BR, Pecher S, Makarenkova V, Shurin MR, Nelson JB. Function and survival of dendritic cells depend on endothelin-1 and endothelin receptor autocrine loops. *Blood*. 2004;104:2107-2115

Appendices:

Figure 1.

Phenotyping of murine dendritic cells (DC) stimulated with $\text{TNF}\alpha$ and treated either with ET_A receptor inhibitor (BQ-123) or ET_B receptor inhibitor (BQ-788). Blockade of ET_A receptors resulted in the decrease of the costimulatory molecule expression, while ET_B receptor blockade was accompanied by mild increase in the expression of costimulatory molecules.

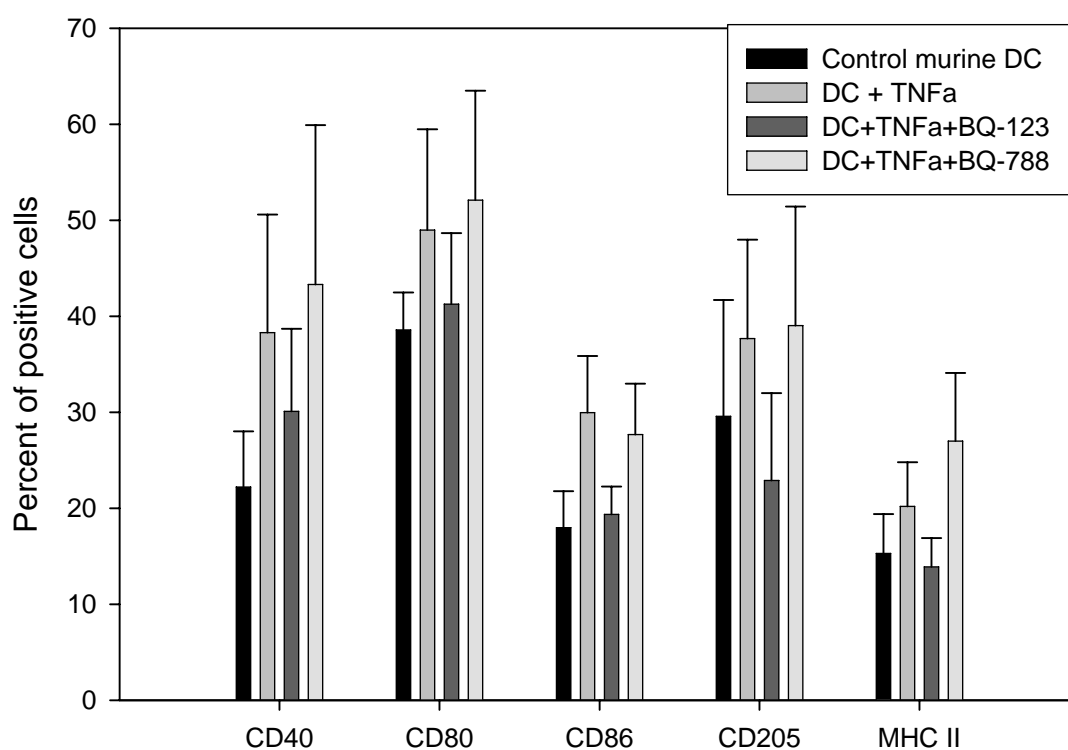


Figure 2.

The influence of the endothelin receptor inhibitors on DC is shown. Briefly, DCs were treated either with TNF α , with TNF α +BQ123 (ET_A receptor inhibitor), and with TNF α +BQ-788 (ET_B receptor inhibitor). Untreated DC provided control. Modified DC were used to stimulate T cells in the mixed leukocyte reaction.

Effect of Endothelin Axis on the Function of Dendritic Cells

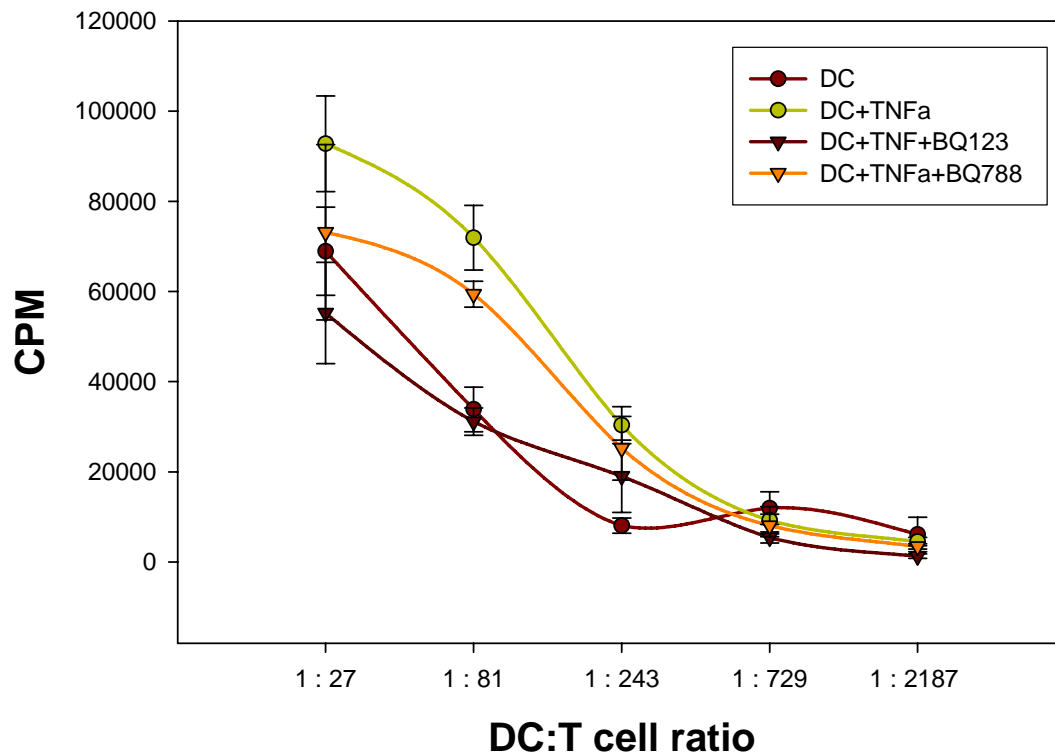


Figure 3.

Dendritic cells were grown in the culture as usual. On days 5 and 6, 1 ml of RM-1 cells (prostate cancer cells) supernatant was added to the culture. Control DC received 1 ml of media. Cells were collected on day 7 and flow cytometry was performed for the expression of co-stimulatory molecules.

Effect of RM-1 prostate cancer cells supernatants on the expression of costimulatory molecules on dendritic cells

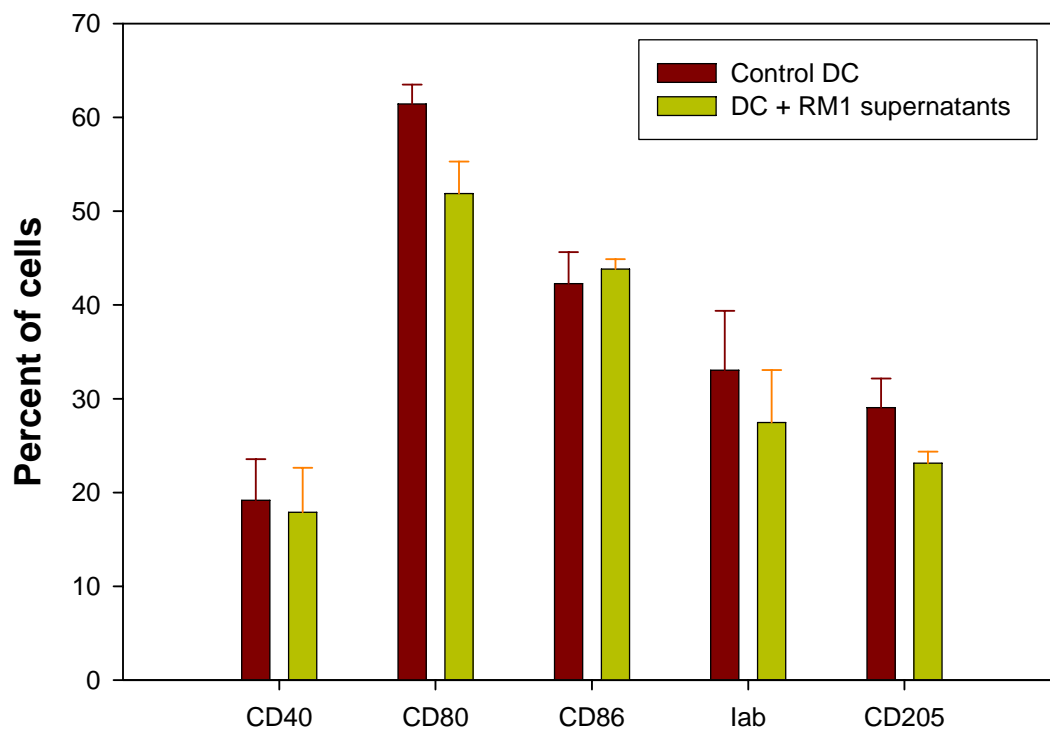


Figure 4.

Mice were injected with RM-1 murine prostate cancer cells. Treatment was started on day 6 by intratumoral injection of the dendritic cells on day 5. Group 1 (control) mice were injected with the vehicle (HBSS), Group 2 - with unmodified DC, Group 3 - with DC treated by $TNF\alpha$, and group 4 - treated with $TNF\alpha$ and BQ-788 (ET_B receptor inhibitor). Tumor size was measured twice a week.

Treatment of mice tumors with modified dendritic cells

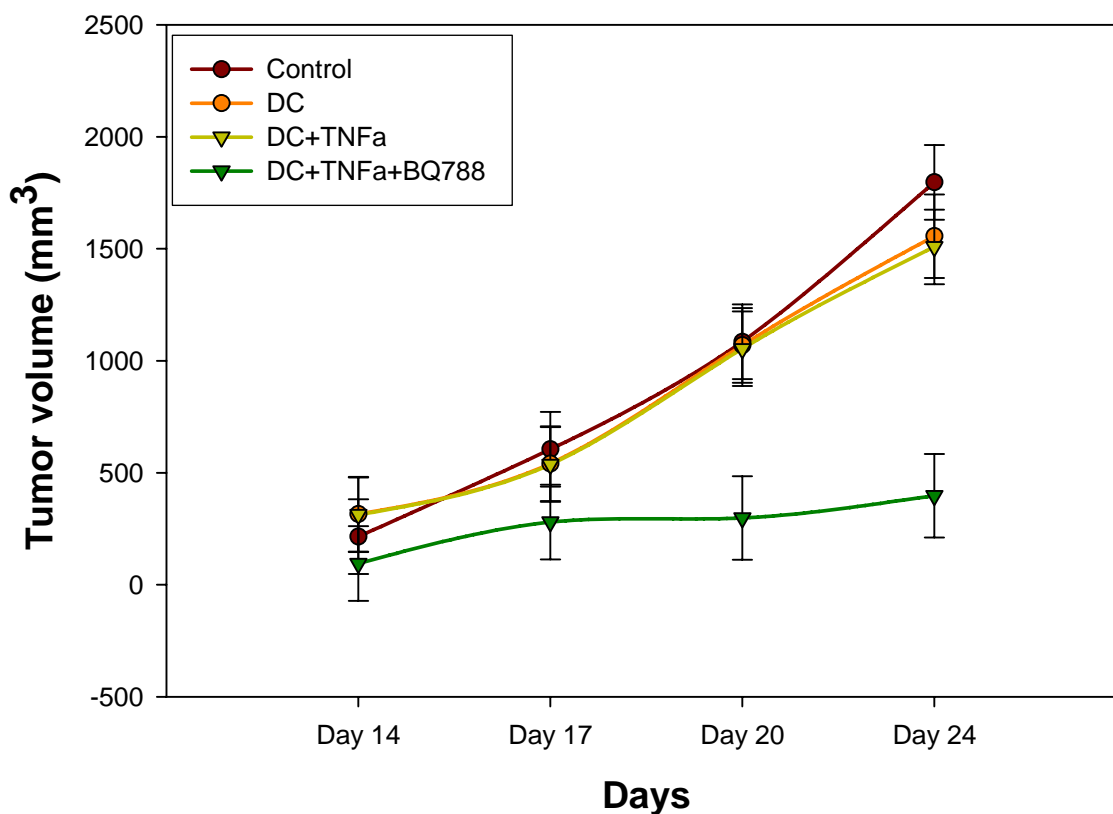
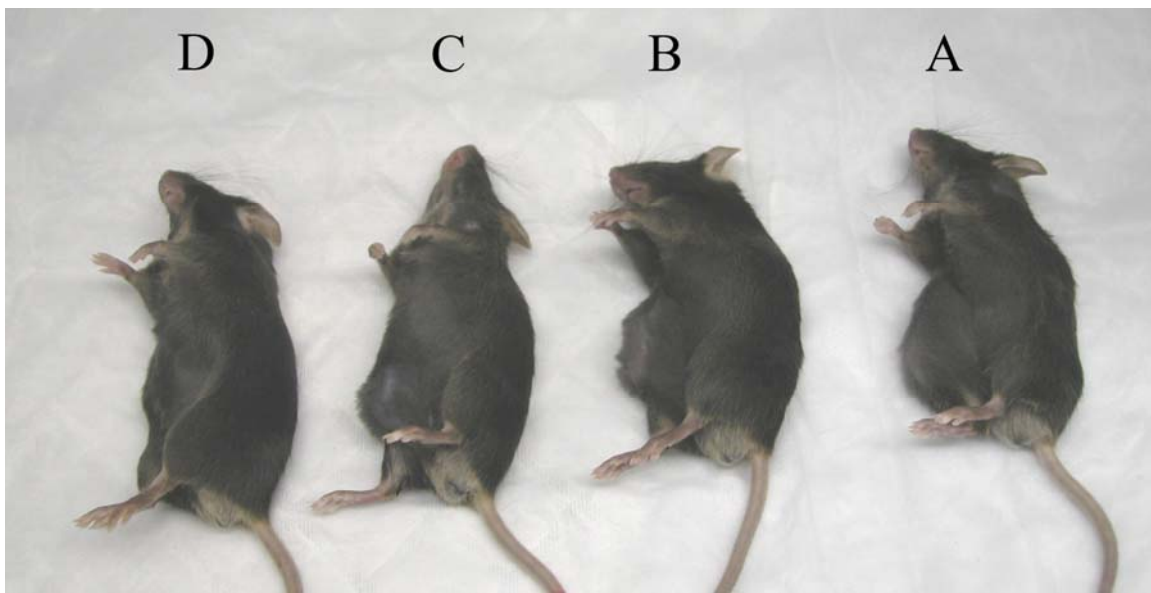


Figure 5.

Mice were injected with RM-1 murine prostate cancer cells. Treatment was started on day 6 by intratumoral injection of the dendritic cells on day 5. Group 1 (control) mice were injected with the vehicle (HBSS), Group 2 - with unmodified DC, Group 3 - with DC treated by $\text{TNF}\alpha$, and group 4 - treated with $\text{TNF}\alpha$ and BQ-788 (ET_B receptor inhibitor). Tumor size was measured twice a week. Pictures of representative mice are presented. A – treated with HBSS, B – treated with DC, C – treated with DC+ $\text{TNF}\alpha$, D – treated with DC+ $\text{TNF}\alpha$ +BQ-788.



Abstract submitted and accepted for the presentation at the AUA 2007 and AACR 2007 Annual Meetings.

Use of modified dendritic cells for the treatment of prostate cancer in mice

Author Block: *Georgi Guruli*, Renee Kancelarich, Peter Hinds, Sean Taheri, Mark L. Jordan, Newark, NJ*

Abstract:

Introduction and Objective:

Immunotherapy via dendritic cells (DC) has shown promise as a novel approach to hormone-refractory prostate cancer. DC are the major antigen-presenting cells and regulators of the immune response. We have previously demonstrated that prostate cancer induces DC apoptosis, which could be one mechanism of tumor escape from immune surveillance. We also demonstrated the presence of endothelin receptors on DC, and that blockade of endothelin B (ET_B) receptors decreases DC apoptosis, while it may enhance their antigen presenting ability. The purpose of the current study was to determine whether ET_B blockade also enhances DC antitumor activity in a murine prostate cancer model.

Methods:

Tumors were induced by subcutaneous injection of 25,000 RM-1 murine prostate cancer cells into groups (n=5) of the C57BL/6 mice. When tumors became palpable (day 6), treatment was initiated with injection of 1.5×10^6 bone marrow derived DC into the tumor. Group 1 received Hank's solution (control); Group 2 – DC grown in the presence of GM-CSF and IL-4 for 7 days; Group 3 – DC prepared as before, with TNF- α added to the culture during the last 48 hours; Group 4 - DC were prepared as in Group 3, but BQ788, ET_B receptor antagonist, was added together with TNF- α (our previous studies have shown the increased expression of endothelin receptors after the stimulation of DC with TNF- α). Injections were repeated 2 more times (total - 3 injections). Approximately 1.5 million cells were injected per mice each time. There were 5 mice per group.

Results:

Serial size was monitored starting from day 3 after the last DC injection, and was repeated every 3 days until the end of the experiment. On day 25, tumor size reached $1796 \pm 166 \text{ mm}^3$ in the Group 1 (control), $1556 \pm 186 \text{ mm}^3$ in the Group 2, $1508 \pm 166 \text{ mm}^3$ in the group 3, and $397 \pm 186 \text{ mm}^3$ in the Group 4. There was statistically significant ($P < 0.001$) reduction of the tumor growth in comparison to control in mice treated with DC modified by ET_B receptor antagonist.

Conclusions:

Protection of DC from apoptosis and counteracting of tolerance may significantly improve their antitumor activity. Because of wide availability of TNF- α and ETB receptor antagonists, these studies can easily be translated into human clinical trials, using autologous dendritic cells.

Author Disclosure Block: G. Guruli, None; R. Kancelarich, None; P. Hinds, None; S. Taheri, None; M.L. Jordan, None.

Keyword - Indexing (Complete): Dendritic cells ; Prostate cancer ; Immunotherapy

Topic (Complete): 39 Basic Research

Source of Funding (Complete):

Funding Source: : Department of Defense - Physician Research Training Grant (GG)
Veterans Administration Merit Review (MLJ)
UMDNJ Foundation
New jersey Medical School Dean's Research Fund

Curriculum Vitae

Name: Georgi Guruli (Pirtskhalaishvili)

Home Address: 93 Locust Avenue
Millburn, NJ 07041

Office Address: Division of Urology
Department of Surgery
UMDNJ – New Jersey Medical School
MSB G-528
185 South Orange Avenue
Newark, NJ 07103
Tel: (973) 972-4488
Fax: (973) 972-3892
Email: gurulige@umdnj.edu,

1. Education

- a. Undergraduate N/A
- b. Graduate

Tbilisi State Medical Institute, Tbilisi, Georgia (former USSR)
Degree: **M.D.**, Date Awarded: June 26, 1983

2. Post Doctoral Training

a. Internships and Residencies

- i. Clinical ordinatura (residency):
Location: Georgian Oncological Research Center, Tbilisi, Georgia
Discipline: Surgical Oncology
Inclusive Dates: 09/1983 – 09/1985.
- ii. Internship (PGY-I):
Location: University of Pittsburgh Medical Center, Pittsburgh, PA
Discipline: Surgery
Inclusive dates: 07/1995 – 06/1996.
- iii. Residency (PGY-II):
Location: University of Pittsburgh Medical Center, Pittsburgh, PA
Discipline: Surgery
Inclusive dates: 07/1996 – 06/1997.
- iv. Residency (PGY-III – PGY-VI):

Location: University of Pittsburgh Medical Center, Pittsburgh,
PA

Discipline: Urology

Inclusive dates: 07/1997 – 06/2001.

b. Research Fellowships

i. Research Fellow:

National Oncological Research Center, Moscow, USSR

Discipline: Urologic Oncology

Inclusive Dates: 09/1987 – 12/1990.

Ph.D. Degree awarded on 12/08/1990.

ii. Research Fellow:

University of Pittsburgh School of Medicine, Pittsburgh, PA

Discipline: Urologic Oncology

Inclusive Dates: 07/2001 – 11/2003.

3. Licensure (state, specialty, issue date, expiration date)

a. Commonwealth of Pennsylvania – Medical Physician and Surgeon,

Initial License Date: 03/05/1999.

b. State of New Jersey – Medical Doctor,

Initial license date: 09/08/2003.

4. Narcotics Certification (state, dates)

CDS registration, Date issued – 10/16/2003.

DEA registration, Date issued – 03/15/2002.

5. University Appointments:

Department: Surgery, Division of Urology

UMDNJ – New Jersey Medical School

Title: Assistant Professor

Inclusive dates: 12/2003 – present.

6. Hospital Appointments

Department of Surgery, Division of Urology

Hospital Name: Georgian Oncological Research Center

Title: Staff physician

Inclusive dates: 1985-1995

Department of Surgery, Division of Urology
University Hospital, Newark
Title: Attending physician
Inclusive dates: 12/2003 – present.

7. Awards and Honors

1977	Gold Medal (Highest Honors), High School #1, Tbilisi, Georgia
1983	Highest Honors (“Red Diploma”), Tbilisi State Medical Institute, Georgia
1998	Second Prize, Clinical Section, Pittsburgh Urological Society Meeting, Pittsburgh, Pennsylvania
1999	Pfizer Scholars in Urology Award.
1999	Best Basic Science Paper Award, 51 st Annual Meeting of Northeastern Section, AUA. Bermuda, UK.
1999	First Prize, Basic Research Section, Pittsburgh Urological Society Meeting, Pittsburgh, Pennsylvania.
2000	Resident Prize Essay Award, 52 nd Annual Meeting of Northeastern Section, AUA. Pittsburgh, USA.
2002	Sylvia Sorkin Greenfield Award, for the best paper published in <i>Medical Physics</i> .
2004	AUA travel Award to attend NIDDK Clinical Research Meeting

8. Board Certification – February 2006 – By the **American Board of Urology**.

9. Principal Clinical and Hospital Service Responsibilities:

Hospital Name: Georgian Oncological Research Center, Tbilisi, Georgia
Department or Service: Urology
Responsibilities – Admission of patients in the hospital, preoperative evaluation and designing of treatment plan, administration of treatment (surgical or medical), postoperative care in the hospital, analyzing treatment outcomes, designing new treatment methods and schemas.
Inclusive Dates: 1985 – 1995.

Hospital Name: University Hospital, Newark, NJ

Department or Service: Surgery (Urology)

Responsibilities – Admission of patients in the hospital, evaluation and elaboration of treatment plan, administration of treatment, post-treatment follow-up, analyzing treatment outcomes, designing and participating in clinical trials.

Inclusive Dates: 12/2003 – present.

10. Ad Hoc Reviewer:

International Journal of Cancer

American Cancer Society

Cytokine

Medical Science Monitor

Grant reviewer for NIH

11. Memberships, Offices and Committee Assignments in professional Societies

i. European Association of Urology

Active Member

1996 – 2000.

ii. American Urological Association

Candidate Member

1997 – 2001

Associate Member

2002 – 2006

Active Member – since 2006

iii. American Association for Cancer Research

Associate Member

Dates: 1999 – 2004.

Active Member – since 2005.

12. Major Research Interests:

b. Prostate cancer:

Relationship and interaction between prostate cancer and dendritic cells (DC), the major antigen-presenting cells. To study the mechanisms of prostate cancer-induced DC suppression, and design the ways of protecting DC from apoptosis. Development of DC-based therapies of advanced prostate cancer.

c. Immunomodulation and the role of endothelin-1 (ET-1) and its receptors in the generation of immune response, in particular, the role of endothelin axis in affecting of DC function.

13. Grant History

a. Principal Investigator

i. Funding Organization: American Foundation for Urologic Disease / American Urological Association Research Scholar
Program Title of Award: The Endothelin Axis: Signaling Pathways

and Maximizing Efficacy in the Treatment of Advanced Prostate Cancer.

Inclusive dates of Funding: 07/2001 – 06/2003.

ii. Funding Organization: Department of Defense, Physician Research Training Grant
Title of Award: Activation and Protection of Dendritic Cells in the Prostate Cancer Environment.

Inclusive dates of Funding: 2005 – 2009.

b. Co-Investigator

i. Funding Organization: University of Pittsburgh Prostate and Urologic Cancer Center Pilot Project (Co-PI)
Title of Award: Effective Protection of Human Dendritic Cells from Prostate Cancer Induced Cell Death.
Inclusive dates of Funding: 1999-2000.

ii. Funding Organization: The Pittsburgh Foundation Program for Medical Research (Co-PI)
Title of Award: New Approach for Prostate Cancer Therapy: Dendritic Cells Protected from Tumor-Induced Death.
Inclusive dates of Funding: 1999-2002.

iii. Funding Organization: Department of Defense (DAMD17-00-1-0099 P1832735, Co-Investigator).
Title of Award: Immune Gene Therapeutic Correction and Protection of Disordered Dendritic Cells in Prostate Cancer.
Inclusive dates of Funding: 1999-2002.

14. Articles

1. Gotsadze, D. T. & **Pirtskhalaishvili, G. G.** (1988). [Diagnosis and treatment of regional metastasis of penile cancer] [Russian]. *Urologiia i Nefrologiia*, 48-51.

2. Gotsadze, D. T., Daneliia, E. V. & **Pirtskhalaishvili, G. G.** (1988). [Lymphogenic metastasis in penile cancer] [Russian]. *Voprosy Onkologii* **34**, 1501-1504.

3. Gotsadze, D. T., Nemsadze, G. G., Chigogidze, T. G., Daneliia, E. V., **Pirtsakhalaishvili, G. G.** & Chovelidze Sh, G. (1990). [A method for forming a large-intestine reservoir for the urine] [Russian]. *Urologiia i Nefrologiia*, 35-39.

4. Gotsadze, D., Mosidze, B., Chigogidze, T., Nemsadze, G., Chovelidze, S. & **Pirtskhalaishvili, G.** (1990). [Surgical aspects for the construction of colonic urinary reservoirs] [Georgian]. *Sakartvelos Sameditsino Moambe*, 36-41.

5. Matveev, B. P., Shipilov, V. I., Gotsadze, D. T., Abdushelishvili, K. O. & **Pirtskhalaishvili, G. G.** (1990). [The incidence of bladder tumor recurrences after transurethral resection during combined treatment] [Russian]. *Urologiia i Nefrologiia*, 53-56.
6. Shipilov, V. I. & **Pirtskhalaishvili, G. G.** (1990). [Transurethral resection in the treatment of locally advanced cancer of the bladder] [Russian]. *Voprosy Onkologii* **36**, 1369-1371.
7. Gotsadze, B. T., Nemsadze, G. G., Mosidze, B. A., **Pirtskhalashvili, G. G.**, Chovelidze Sh, G. & Daneliia, E. V. (1991). [A "dry" abdominal urinostoma] [Russian]. *Vestnik Khirurgii Imeni i - i - Grekova* **146**, 120-122.
8. Matveev, B. P., Gotsadze, D. T. & **Pirtskhalaishvili, G. G.** (1991). [The results of cystectomy in bladder cancer] [Russian]. *Voprosy Onkologii* **37**, 1095-1098.
9. Gotsadze, D. T., Daneliia, E. V., **Pirtskhalaishvili, G. G.** & Arutiunov, E. T. (1991). [Malignant tumors of the testis in the Georgian SSR] [Russian]. *Voprosy Onkologii* **37**, 25-28.
10. Gotsadze, D., **Pirtskhalaishvili, G.**, Danelia, E., Chovelidze, S., Zangaladze, L. & Zedginidze, T. (1991). [Abdominal reservoir as an alternative to cutaneous urinary diversion] [Russian]. *Diagnosis and treatment of genitourinary tumors*. B. P. Matveev (Ed.). Moscow: 54-59.
11. Daneliia, E. V., Gotsadze, D. T. & **Pirtskhalaishvili, G. G.** (1992). [The lack of knowledgeability of men about testicular tumors as a cause for the late diagnosis of this disease] [Russian]. *Voprosy Onkologii* **38**, 1254-1258.
12. Gotsadze, D. T. & **Pirtskhalaishvili, G. G.** (1992). [The quality of life of patients after cystectomy for cancer] [Russian]. *Voprosy Onkologii* **38**, 489-493.
13. Matveev, B. P., Gotsadze, D. T. & **Pirtskhalaishvili, G. G.** (1993). [The causes of mortality following cystectomy for bladder tumor] [Russian]. *Urologiia i Nefrologiia*, 20-22.
14. Gotsadze, D. T., **Pirtskhalaishvili, G. G.**, Chovelidze Sh, G. & Chigogidze, T. G. (1993). [The results of the diversion of urine into a large-intestine reservoir] [Russian]. *Urologiia i Nefrologiia*, 28-30.
15. Gotsadze, D., Charkviani, L., Nemsadze, G., Tsintsadze, I. & **Pirtskhalaishvili, G.** (1994). Continent urinary diversion (Gotsadze Pouch) after pelvic exenteration for gynaecological malignancies. *European Journal of Gynaecological Oncology* **15**, 369-371.

16. Gotsadze, D. T., **Pirtskhalaishvili, G. G.** & Alkhanishvili, K. B. (1995). [A detubularized reservoir for the urine made from the small intestine] [Russian]. *Urologiia i Nefrologiia*, 38-41.

17. Gotsadze, D. & **Pirtskhalaishvili, G.** (1995). Abdominal reservoirs for continent urinary diversion. *Journal of Urology* **154**, 985-988.

18. Gotsadze, D. T., **Pirtskhalaishvili, G. G.** & Alkhanishvili, K. B. (1996). [The choice of urinary diversion in tumors of the small pelvis] [Russian]. *Voprosy Onkologii* **42**, 82-84.

19. Gotsadze, D. & **Pirtskhalaishvili, G.** (1998). Meckel's diverticulum as a continence mechanism. *Journal of Urology* **160**, 831-832.

20. **Pirtskhalaishvili, G.**, Konety, B. R. & Getzenberg, R. H. (1999). Update on urine-based markers for bladder cancer. How sensitive and specific are the new noninvasive tests? *Postgraduate Medicine* **106**, 85-86, 91-94.

21. **Pirtskhalaishvili, G.**, Getzenberg, R. H. & Konety, B. R. (1999). Use of urine-based markers for detection and monitoring of bladder cancer. *Techniques in Urology* **5**, 179-184.

22. **Pirtskhalaishvili, G.** & Shurin, M. R. (2000). Dendritic cells in the treatment of prostate cancer. *Cancer Research Alert* **1**, 89-91.

23. **Pirtskhalaishvili, G.**, Shurin, G. V., Esche, C., Cai, Q., Salup, R. R., Bykovskaya, S., Lotze, M. T. & Shurin, M. R. (2000). Cytokine-mediated protection of human dendritic cells from prostate cancer-induced apoptosis is regulated by the Bcl-2 family of proteins. *British Journal of Cancer* **83**, 506-513.

24. **Pirtskhalaishvili, G.** & Nelson, J. B. (2000). Endothelium-derived factors as paracrine mediators of prostate cancer progression. *Prostate* **44**, 77-87.

25. **Pirtskhalaishvili, G.**, Shurin, G. V., Gambotto, A., Esche, C., Wahl, M., Yurkovetsky, Z. R., Robbins, P. D. & Shurin, M. R. (2000). Transduction of dendritic cells with Bcl-xL increases their resistance to prostate cancer-induced apoptosis and antitumor effect in mice. *Journal of Immunology* **165**, 1956-1964.

26. Esche, C., Shurin, G. V., Kirkwood, J. M., Wang, G.-Q., Rabinowich, H., **Pirtskhalaishvili, G.** & Shurin, M. R. (2001). TNF- α -promoted expression of Bcl-2 and inhibition of mitochondrial cytochrome C release mediate resistance of mature dendritic cells to melanoma-induced apoptosis. *Clinical Cancer Research* **7**, 974s-979s.

27. Konety, B.R., Lavelle, J.P., Pirtskhalaishvili, G., Dhir, R., Meyers, S., Nguyen T.-S.T., Hershberger, P., Shurin, M.R., Johnson, C.S., Zeidel, M.L., Getzenberg, R.H.

(2001) Effects of Vitamin D (calcitriol) on transitional cell carcinoma of the bladder in vitro and in vivo. *Journal of Urology* **165**, 253-258.

28. **Pirtskhalaishvili, G.**, Shurin, G. V., Esche, C., Trump, D. L. & Shurin, M. R. (2001). TNF- protects dendritic cells from prostate cancer-induced apoptosis. *Prostate cancer and prostatic diseases* **4**, 221-227.

29. **Pirtskhalaishvili, G.**, Hrebinko, R. L. & Nelson, J. B. (2001). The treatment of prostate cancer: an overview of current options. *Cancer Practice* **9**, 295-306.

30. Pan, Y., Lavelle, J. P., Bastacky, S. I., Meyers, S., **Pirtskhalaishvili, G.**, Zeidel, M. L. & Farkas, D. L. (2001). Detection of tumorigenesis in rat bladders with optical coherence tomography. *Medical Physics* **28**, 2432-2440.

31. Shurin, G. V., Aalamian, M., **Pirtskhalaishvili, G.**, Bykovskaia, S., Huland, E., Huland, H. & Shurin, M. R. (2001). Human prostate cancer blocks the generation of dendritic cells from CD34+ hematopoietic progenitors. *European Urology* **39 Suppl 4**, 37-40.

32. **Pirtskhalaishvili, G.** & Nelson, J. B. (2002). The Endothelin Receptor: A Novel Target for Anticancer Therapy. *American Journal of Cancer* **1**, 81-91.

33. Konety B.R., **Pirtskhalaishvili G.** (2002) Transitional cell carcinoma, renal. In: Cunha BA, Geibel J, Leslie SW, Marriott HJL, Schulman P, Shulman LP, Soreff S, Zevitz ME, eds. *Medicine, OB/Gyn, Psychiatry, and Surgery - An online medical reference*. Vol. 3: Emedicine Journal; <http://www.emedicine.com/med/topic2003.htm> (Updated in 2006).

34. Makarenkova V.P., Shurin G.V., Tourkova I.L., Balkir L., **Pirtskhalaishvili G.**, Perez L., Gerein V., Siegfried J.M., Shurin M.R. (2003) Lung cancer-derived bombesin-like peptides down-regulate the generation and function of human dendritic cells. *Journal of Neuroimmunology* **145**:55-67

35. **Guruli G.**, Pflug B.R., Pecher S., Makarenkova V., Shurin M.R., Nelson J.B. (2004) Function and survival of dendritic cells depend on endothelin-1 and endothelin receptor autocrine loops. *Blood* **104**: 2107-15.

36. Nelson J.B., Udan M.S., **Guruli G.** and Pflug B.R. (2005) Endothelin-1 Inhibits Apoptosis in Prostate Cancer; *Neoplasia* **7**: 631-637.

37. Akhavan A., McHugh K.H., **Guruli G.**, Bies R.R., Zamboni W.C., Strychor S.A., Nelson J.B., Pflug B.R. (2006) Endothelin receptor A blockade enhances taxane effects in prostate cancer. *Neoplasia*. **8**:725-732.

15. Books, Monographs and Chapters

1. Gotsadze D, Mosidze B, Nemsadze G, Chovelidze S, **Pirtskhalaishvili G.** [Construction of colonic reservoir for urinary diversion] [Russian]. *Methodical Recommendations*. Tbilisi; 1990.
2. **Pirtskhalaishvili G**, Nelson JB. Endothelins. In: Creighton T, ed. *Encyclopedia of Molecular Medicine*. New York: John Wiley & Sons; 2002.
3. Zeidel ML, **Pirtskhalaishvili G.** Urinary tract obstruction. In: Brenner MB, ed. *The Kidney*. Vol. 2 (ed 7th). Philadelphia: Saunders; 2004:1867-1893

16. Abstracts

1. Gotsadze, D., **Pirtskhalaishvili, G.**, Chovelidze, S. & Nemsadze, G. (1992). Indications for construction of artificial valve for colonic reservoirs. In *Proceedings of Continent Urinary Reconstruction*. First International Meeting, Lund, Sweden. 10-12 June 1992 (p. 149). Scandinavian Journal of Urology and Nephrology, Suppl.
2. Gotsadze, D. T., Nemsadze, G. G., Gvamichava, R. R. & **Pirtskhalaishvili, G.** (1994). Dry umbilical urinostoma after pelvic exenterative surgery. In *2nd International conference on Colo-Rectal Tumours*, Milan, Italy. 11-14 September 1994 (p. F3).
3. Gotsadze, D., Chovelidze, S. & **Pirtskhalaishvili, G.** (1994). Combined cytoreductive organ-spared surgical treatment in patients requiring cystectomy. In *All-Russian Conference on Oncourology*, Obninsk, Russia. 6-7 October 1994 (pp. 63-64).
4. Gotsadze, D., Charkviani, L., Nemsadze, G., Tsintsadze, I., **Pirtskhalaishvili, G.** & Alkhanishvili, K. (1995). Ileal urinary reservoir (Gotsadze Pouch) after exenteration for gynaecological malignancies. In *9th International Meeting of Gynaecological Oncology (ESGO)*, Knokke, Belgium. 9-12 May 1995 (p. 63). European Society of Gynaecological Oncology.
5. **Pirtskhalaishvili, G.** & Hrebinko, R. L. (1998). The prognostic utility of prostate specific antigen density (PSAD). In *50th Anniversary Meeting of the Northeastern Section, American Urological Association*, Toronto, Canada. 18-21 October 1998 (p. 132). Northeastern section, American Urological Association.
6. Hrebinko, R. L. & **Pirtskhalaishvili, G.** (1998). Reliability of ultrasound-determined prostate volumes. In *50th Anniversary Meeting of the Northeastern Section, American Urological Association*, Toronto, Canada. 18-21 October 1998 (p. 140). Northeastern section, American Urological Association.

7. **Pirtskhalaishvili, G.**, Hrebinko, R. L., Robbins, P., Gambotto, A., Lotze, M. T. & Shurin, M. R. (1999). Intratumoral injection of dendritic cells protected from tumor-induced apoptosis causes inhibition of prostate cancer growth in mice. In *Joint Annual Meeting of the New England and Northeastern Sections of the American Urological Association*, Southampton, Bermuda. 31 October - 4 November 1999 (p. 200). Northeastern section, American Urological Association.
8. Konety, B., **Pirtskalashvili, G.**, Hersherberger, P., Johnson, C. & Getzenberg, R. (1999). Vitamin D induced apoptosis of bladder tumor cells in vitro. In *AUA 94th Annual Meeting*, Dallas, Texas. 1-6 May 1999 (p. 119). American Urological Association.
9. **Pirtskhalaishvili, G.**, Salup, R. R., Esche, C., Lotze, M. & Shurin, M. R. (1999). Protection of Human dendritic cells from prostate cancer-induced apoptosis by IL-12 and IL-15. In *94th Annual Meeting of the American Urological Association*, Dallas, Texas. 1-6 May 1999 (p. 126). American Urological Association.
10. **Pirtskhalaishvili, G.**, Salup, R. R., Esche, C., Lotze, M. T. & Shurin, M. R. (1999). Induction of apoptosis in human dendritic cells by prostate cancer and the mechanisms of protection. In *90th Annual Meeting of the American Association for Cancer Research*, Philadelphia, Pennsylvania. 10-14 April 1999 (p. 322). American Association for Cancer Research.
11. Salup, R. R., **Pirtskhalaishvili, G.**, Deng, D. H., Batthacharya, R., Tran, S. & Lotze, M. T. (1999). Vaccination with plasmid DNA encoding for a truncated HER2/neu protein prevents the growth of prostate cancer in rats and induces a tumor antigen specific immune response in vivo. In *90th Annual Meeting of the American Association for Cancer Research*, Philadelphia. 10-14 April 1999 (p. 256). American Association for Cancer Research.
12. Konety, B. R., Lavelle, J. P., **Pirtskhalaishvili, G.**, Calleary, J. G., Meyers, S. A., Ramage, R., Dhir, R., Zeidel, M. L. & Getzenberg, R. H. (2000). Evaluation of vitamin D in the prevention and treatment of bladder cancer. In *95th Annual Meeting of the American Urological Association*, Atlanta, Georgia. 29 April - 4 May 2000 (p. 120). American Urological Association.
13. **Pirtskhalaishvili, G.**, Gambotto, A., Esche, C., Yurkovetsky, Z. R., Lotze, M. R. & M.R., S. (2000). IL-12 and Bcl-x_L gene transfection of murine dendritic cells protects them from prostate-cancer induced apoptosis and improves their antitumor activity. In *95th Annual Meeting of the American Urological Association*, Atlanta, Georgia. 29 April - 4 May 2000 (p. 105). American Urological Association.

14. **Pirtskhalaishvili, G.**, Gambotto, A., Yamabe, K., Lotze, M. T. & Shurin, M. R. (2000). Protection of dendritic cells (DC) from tumor-induced apoptosis increases the efficacy of DC-based therapy in a murine prostate cancer model. In *91st Annual Meeting of the American Association for Cancer Research*, San-Francisco, California. 1-5 April 2000 (p. 43). American Association for Cancer Research.

15. **Pirtskhalaishvili, G.**, Lavelle, J. P., Callear, J. G., Meyers, S. A., Ramage, R., Bastacky, S. I. & Zeidel, M. L. (2000). Physiological properties of the urothelium during methyl-nitroso-urea (MNU) induced carcinogenesis. In *52nd Annual Meeting, The Northeastern Section of the AUA*, Pittsburgh, Pennsylvania. 14-17 September 2000 (p.140). Northeastern Section, American Urological Association.

16. Bhattacharaya, R., Prawoko, I., Morgan, M., **Pirtskhalaishvili, G.**, Soto, J. & Salup, R. R. (2001). Vaccination with plasmid DNA encoding for HER2/neu-EGFP fusion protein immunizes rats against prostate cancer. In *92nd Annual Meeting of the American Association for Cancer Research*, New Orleans, Louisiana. 24-28 March 2001 (p. 819). American Association for Cancer Research.

17. Aalamian, M., **Pirtskhalaishvili, G.**, Yamabe, K., Esche, C., Shurin, G. V., Huland, E., Huland, H. & Shurin, M. R. (2002). Prostate cancer suppresses dendropoiesis both in vitro and in vivo. In *AUA 97th Annual Meeting*, Orlando, Florida. 25-30 May 2002 (p. 136). American Urological Association.

18. **Pirtskhalaishvili, G.**, M.athis, C., Arlotti, J. A., Pflug, B. R. & Nelson, J. B. (2002). PET imaging of prostate cancer through manipulation of fatty acid synthase. In *AUA 97th Annual Meeting*, Orlando, Florida. 25-30 May 2002 (p. 362). American Urological Association.

19. Shurin, M. R., **Pirtskhalaishvili, G.**, Tourkova, I. L., Yurkovetsky, Z. R., Perez, L., Makarenkova, V. P., Balkir, L. & Shurin, G. V. (2002). The dendritic cell system under tumor surveillance. In *The 2nd International Conference on Tumor Microenvironment: Progression, Therapy and Prevention*, Baden, Austria. 25-29 June 2002 (p.94).

20. **Pirtskhalaishvili, G.**, Pflug, B. R., Pecher, S., Makarenkova, V., Shurin, M. R. & Nelson, J. B. (2003). Presence of an endothelin-1/ETA autocrine loop in the maturation, differentiation and survival of human dendritic cells. In *94th Annual Meeting of the American Association for Cancer Research*, Washington, DC. 11-14 July 2003 (p. 555). American Association for Cancer Research.

21. **Guruli, G.**, Pflug, B.R., Shurin, M. R. and Nelson, J. B.: Gene expression changes in dendritic cells after their interaction with prostate cancer cells. In *96th*

Annual Meeting of the American Association for Cancer Research, Anaheim, CA, April 16-20, 2005 (Abstract #4563), at 96th Annual Meeting of AACR.

17. Patents held:

Title: **“Endothelin Axis and the Action of Dendritic Cells”**
University of Pittsburgh Case No. 00743
U.S. Patent Number: **U.S. Patent Application No. 60/513,729**
Date of Issue: pending

18. Languages spoken: Georgian, English, Russian